4-Pyridazinecarboxylic acid is reported to melt at 239–240° with decomposition.[2b]

3-Butylpyridazine. To a solution of 8.0 g. of pyridazine in 500 ml. of ether was added (30 min.) 246 ml. of 0.477 *M* *n*-butyllithium (0.116 mole). The temperature was maintained at −15°. After 30 minutes stirring, the mixture was allowed to warm to room temperature overnight, then was hydrolyzed with water. Extraction of the aqueous layer with ether and distillation of the organic portion yielded 9.15 g. (66.3% calculated as butyldihydropyridazine) of product; b.p. 61.5–66.5° (0.5 mm.); $n_D^{25.2}$ 1.4940. The infrared spectra, in contrast to that for the 4-butylpyridazine obtained by the previously described reaction, showed a strong absorption peak at 3.02 microns, no strong peak at 6.30 microns, and the 7–12 region was unlike that of the pyridazines. These data indicate that the product was the dihydro compound, as confirmed by the analysis.

Anal. Calc'd for $C_8H_{14}N_2$: C, 69.52; H, 10.21. Found: C, 69.98; H, 10.17.

The dihydro compound (3 g.) was oxidized with 2.29 g. of potassium permanganate in 450 ml. of acetone at a temperature which did not exceed 15°. Distillation of the organic products gave 0.25 g. b.p. 77–79.5° (1 mm.) and 2.08 g., b.p. 79.5–82° (1 mm.); $n^{25.2}$ 1.4950 [total yield b.p. 77–82° (1 mm.), 2.33 g. (77%)].

Anal. Calc'd for $C_8H_{12}N_2$: C, 70.55; H, 8.89. Found: C, 71.15; H, 8.91.

No crystalline picrate could be obtained from this material. The infrared spectra was characteristic of a pyridazine. Absorption at 3.12 microns was weak and there were good peaks at 6.30 and 6.98 microns. Furthermore, the 7–12 region resembled that of 3-methylpyridazine.

A 2.00-g. sample of the butylpyridazine was oxidized under the conditions used for the 4-butylpyridazine. In this case, a 32% yield of 3-pyridazinecarboxylic acid, m.p. 199–200° (dec.), was obtained as the only isolable acid. On recrystallization from water, 0.3 g. of acid was isolated which melted with decomposition at 203.5–204.5°; the melting point reported for 3-pyridazinecarboxylic acid is 201° (with decomposition).[2c]

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Degradation of Cortisol-C^{14} and Corticosterone-C^{14} Biosynthesized from Acetate-1-C^{14} [1,2]

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Acetate is known to be a precursor of cholesterol and of various steroid hormones. The arrangement of the majority of carbons incorporated into the cholesterol molecule from acetate is known with certainty.[3–6] On the basis of these findings together with the observation that squalene may be converted to cholesterol, Woodward and Bloch[5] postulated the arrangement of methyl and carboxyl carbons derived from acetate in the cholesterol molecule. This communication is concerned with the arrangement of some of the methyl and carboxyl carbons in cortisol and corticosterone derived by biosynthesis from acetate-1-C^{14}.

EXPERIMENTAL

Calf adrenal glands were perfused with a glucose-fortified, physiologically balanced and buffered salt solution containing acetate-1-C^{14} and gased with 95% O_2/5% CO_2 as described by Rosenfeld.[7] Two perfusions were done, one using 3 mc and the other 5 mc of acetate-1-C^{14}. In each case the glands were perfused for two hours at 37.5° in a multicycle system with one liter of fluid.

The perfusates were extracted three times with two volumes of isopropyl acetate. The extracts were washed with a saturated solution of sodium bicarbonate and water until neutral. The isopropyl acetate extract was dried over sodium sulfate and evaporated *in vacuo* to yield a neutral residue containing 2.62×10^6 counts per minute (c/m). The sodium bicarbonate solution was acidified with hydrochloric acid, extracted with isopropyl acetate, washed with water, dried over sodium sulfate, and evaporated *in vacuo* to yield an acidic residue containing 0.5×10^6 c/m.

Carrier cortisol (5 mg.) and corticosterone (5 mg.) were added to the neutral residue which then was chromatographed on paper in the toluene-propylene glycol system (TPG) for 78 hours. The run off was rechromatographed for 16 hours in the TPG system. Cortisol and corticosterone were detected by radioautography, scanning under the ultraviolet lamp, and the blue tetrazolium reaction. After each compound was rechromatographed twice on paper, single spots were detected. The eluted material then was chromatographed on a partition column using ethanol-water (1:3) as the stationary phase on a Celite support and benzene as the mobile phase. The hormones, which came off the columns as single peaks, were further diluted with nonradioactive steroids and were recrystallized ten times to a constant specific activity. The C^{14}-labeled hormones, of constant specific activity, were combusted and were counted as carbon dioxide. A total of 192 mg. of cortisol-C^{14} of 0.525×10^6 disintegrations per minute per millimole (d/m/mM) and 190 mg. of corticosterone-C^{14} of 0.635×10^6 d/m/mM were obtained.

The ketolic side chain of cortisol-C^{14} was cleaved with sodium bismuthate[8,9] and C-21 was isolated as the formadone and C-20 as barium carbonate. Corticosterone was similarly reacted with sodium bismuthate and C-21 again was isolated as the formadone. The products were counted as carbon dioxide. No C^{14} could be detected in C-21 derived from either cortisol or corticosterone. The sensitivity of the counting procedure would permit the finding of 3880 d/m/mM for the cortisol carbon and 5250 d/m/mM for the corticosterone

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(2) The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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carbon. The barium carbonate derived from C-20 of the biosynthesized cortisol-C^{14} contained 48,000 d/m/mM.

On the basis of the scheme postulated by Woodward and Bloch[5] one would expect ten radioactive carbons to be incorporated into the first 21 carbons of cholesterol from acetate-1-C^{14}. If one assumes that corticosteroids are biosynthesized from acetate-1-C^{14} through cholesterol then the arrangement of all carbons and the number of C^{14} atoms in corticosteroids would be expected to be the same as that found in the first 21 carbons of cholesterol. Our findings demonstrate the absence of radioactivity in carbons 21 of both cortisol-C^{14} and corticosterone-C^{14}. By our method of counting we can say that if radioactivity was present in C-21 of cortisol the value must be less than 8.1% of that found in C-20. The count of 48,000 d/m/mM. found in C-20 of cortisol-C^{14} is in reasonable agreement with the calculated value of 52,500 d/m/mM. Our findings demonstrate that the arrangement of methyl and carboxyl carbons in the corticoid side chain is identical to that of carbons 20 and 21 of cholesterol.

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The Preparation of 3,4-Bis(2-furyl)-1,2,5-oxadiazole[1,2]

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INTRODUCTION

A compound, later identified as 3,4-bis(2-furyl)-1,2,5-oxadiazole, precipitated gradually from urea solutions of α-furildioxime prepared in connection with a study of the effect of urea on the solubility of water-soluble *vic*-dioximes. 3,4-Bis(2-furyl)-1,2,5-oxadiazole was characterized by Tsumaki and Yamaguchi[3] who originally obtained it as a product of the pyrolysis of bis(α-furildioximato-N,N′)-nickel(II).

3,4-Bis(2-furyl)-1,2,5-oxadiazole has been prepared in 58% yield by treating α-furildioxime with urea solution. This compound has been prepared also from both the α- and γ-furildioxime by modifying the conditions of a sealed tube reaction described by Auwers and Meyer[4] for the preparation of diphenylfurazan.

The effect of urea on the aqueous solubility of 2,3-butanedionedioxime, 1,2-diaminoethanedionedioxime, 3-methyl-, 4-methyl-, 3-ethyl-, 4-isopropyl-, and 4-*tert*-amyl-1,2-cyclohexanedionedioxime, 1,2-cycloheptanedionedioxime, 1,2-cyclohexanedionedioxime and α-benzildioxime was also studied. This catalytic dehydration by urea does not seem to be a general reaction since none of the above aliphatic, alicyclic or aromatic *vic*-dioximes were converted to the corresponding 1,2,5-oxadiazoles by urea under the conditions found most satisfactory for preparing 3,4-bis(2-furyl)-1,2,5-oxadiazole.

EXPERIMENTAL WORK

From α-furildioxime. α-Furildioxime, 1.2556 g. (0.0057 mole) was treated with 25 ml. of 50% (w/w) urea solution, and the mixture was warmed to 80°. The solution soon became discolored and cloudy, and an oily phase appeared. Heating was continued until the aqueous phase became clear. As the mixture cooled, the 3,4-bis(2-furyl)-1,2,5-oxadiazole solidified and was collected by filtration. This material was dissolved in the minimum amount of methanol and was precipitated by the addition of 150 ml. of distilled water. The yield of 3,4-bis(2-furyl)-1,2,5-oxadiazole, m.p. 61–62°, was 0.6602 g. (58%).

α-Furildioxime, 1.3625 g. (0.006 mole) and water, 2.5 g. (0.14 mole), were sealed in a 200-ml. container constructed from borosilicate tubing ($1^3/_4$-inch O.D., $^3/_{32}$-inch wall thickness). The container then was placed in a 12-inch section of 2-inch iron pipe closed at both ends with pipe caps. The container was heated in an oven at 175° for 8 hours. Upon cooling, the container was opened, and the contents were rinsed out with methanol. The methanolic solution of the product was heated, filtered, and concentrated by evaporation to a volume of 5 ml. Crystallization of the 3,4-bis(2-furyl)-1,2,5-oxadiazole was effected by the addition, with swirling, of 150 ml. of distilled water. The yield was 0.1515 g. (12%).

From γ-furildioxime. 3,4-Bis(2-furyl)-1,2,5-oxadiazole was obtained by heating γ-furildioxime with water in a sealed container for 12 hours in an oven at 165°. The amount of water necessary to produce a pressure of 25 atmospheres was calculated from the simple gas law. The product was purified by the previously described procedure. The yield from this isomer of the dioxime was 20.5%.

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Phenylboronates of Pentoses and 6-Deoxyhexoses

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Although it is known that boric acid and boric acid derivatives react with polyhydric alcohols and with sugars, stable products have not been isolated from the reducing sugars.[2–4] Kuivila and coworkers[4] have described the phenylboronates, a new

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